Levels of Oxidative Metabolites, Antioxidants and Neopterin in Nigerian Pulmonary Tuberculosis Patients

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ABSTRACT

***Aim**: Several products of cellular (macrophage) activation (i.e. free radicals and cytokines) are potential causes of metabolic disorders in pulmonary tuberculosis (PTB) patients. There is a dearth of information on the levels of neopterin and oxidative metabolites in Nigerian PTB patients. This study therefore assessed the levels of markers of oxidative stress and neopterin in Nigerian PTB patients.*

***Method**: Thirty-eight (17 males and 21 females) newly diagnosed PTB-patients and 40 (22 males and 18 females) apparently healthy non-PTB controls volunteered to participate in this study. Diagnostic criteria included sputum acid fast bacilli, chest X-ray and Mantoux test. The levels of superoxide dismutase, plasma albumin, total antioxidant potential, nitric oxide, reduced glutathione, catalase, total plasma peroxide, oxidative stress index, malondialdehyde and neopterin (marker of cellular activation) were determined in them using spectrophotometric methods and enzyme linked immunosorbent assay (ELISA) technique respectively.*

***Result**: In PTB-patients, significantly ($p<0.05$) lower levels of albumin, superoxide dismutase, reduced glutathione, catalase, nitric oxide and total antioxidant potential with significantly ($p<0.05$) higher levels of total plasma peroxide, malondialdehyde, oxidative stress index and neopterin were observed when compared with the non-PTB controls. The result indicates significantly higher levels of cellular activation, free radical load and oxidative stress with associated antioxidants depletion in PTB patients.*

***Conclusion**: Oxidative stress and increased level of neopterin are features of pulmonary tuberculosis. Since most of the antioxidants regulating the free radical load and oxidative stress are micronutrient dependent, micronutrient supplementation may be required as adjuvant therapy in the management of PTB patients.*

***Key words**: Nigeria, oxidative stress, neopterin, pulmonary tuberculosis*

Nijeryalı Akciğer Tüberkülozu Hastalarındaki Oksidatif Metabolitler, Antioksidanlar ve Neopterin Düzeyleri

Amaç: *Birçok hücresel (makrofaj) aktivasyon (ör: serbest radikal ve sitokinler) ürünleri akciğer tüberkülozunda potansiyel metabolic bozukluk sebebidir. Nijeryalı ATB hastalarında nepterin ve oksidatif stress seviyeleri konusunda bilgi kısıtlılığı mevcuttur. Bu nedenle bu çalışma Nijeryalı ATB hastalarında oksidatif stress ve neopterin belirteçlerini değerlendirdi.*
Metod: *Otuz sekiz (17 erkek, 21 kadın) yeni tanı ATB hastası ve 40 (22 erkek ve 18 kadın) sağlıklı ATB olmayan göünüllü controller çalışmaya katıldı. Tanı kriterleri balgamda ARB, göğüs radyografisi ve Mantoux testini içermekteydi. Süperoksit dismutaz, plazma albumin, total antioksidan potasiyeli, nitric oksit, indirgenmiş glutatyon, total plazma peroksit, oksidatif stress indeksi, malonildialdehit ve neopterin(hücresel aktivasyon belirteci) seviyeleri spektrofotometrik metodlar ve enzyme linked immunosorbent assay (ELISA) tekniği ile ayrı ayrı belirlendi.*
Bulgular: *ATB hastalarında ATB olmayan kontrollere gore albumin, süperoksit dismutaz, indirgenmiş glutatyon, katalaz, nitrikoksit ve total antioksidan potansiyeli seviyeleri anlamlı olarak düşük ($p<0.05$);plazma peroksit, malonildialdehit, oksidatif stress indeksi ve neopterin seviyeleri anlamlı olarak yüksek ($p<0.05$) izlendi. Sonuçlar ATB hastalarında, antioksidan azlığı ile ilişkili hücresel aktivasyon, serbest radikal yükü ve oksidatif stress seviyelerinde anlamlı yükseklik göstermektedir.*
Sonuç: *Oksidatif stress ve yüksek neopterin seviyesi akciğer tüberkülozunun özelliğidir. Antioksidanların mikrobesin bağımlı serbest radikal yükünü ve oksidatif stresi düzenlemesinden dolayı, mikrobesin takviyesi ATB hastaları için adjuvan tedavi olarak gerekli olabilir.*
Anahtar kelimeler: *Nijerya, oksidatif stres, neopterin, akciğer tüberkülozu*

INTRODUCTION

The tubercle bacilli invaded-macrophage undergoes intracellular transformation to generate reactive nitrogen intermediate (RNI) and reactive oxygen species (1, 2). The purpose of the free radical generation is to keep the tubercle bacilli in a latent form through the synergistic effect of interferon-γ and the reactive nitrogen intermediate (RNI) (1, 2). Several authors reported that animals lacking inducible nitric oxide synthase were found to be more susceptible to M. tuberculosis and Leishmania major infections (3). Cellular immune deficiency and decreased NO synthesis (through malnutrition and other factors) enhance the progression of the latent tuberculosis to active pulmonary tuberculosis (2).

Active pulmonary tuberculosis (PTB) is characterized by increased rate of macrophage activation and oxygen uptake of the activated macrophages above the baseline levels. The increased oxygen uptake in the presence of NADPH oxidase is converted to superoxide anion and other free radicals (2). When the free radical load exceeds the detoxification capacity of the endogenous antioxidant defenses, it results to oxidative stress (4). The consequences of oxidative stress in disease conditions include fragmentation of proteins and peroxidation of lipids, dysfunction of cell membranes and enzymes, impairment of cell membrane function, decreased fluidity, inactivation of membrane-bound receptors, and increased permeability of ions (2).

Neopterin is a metabolic product of macrophage activation. It is an indicator of pro-inflammatory immune status and a marker of cellular activation that is released into the circulation (6). Several researchers have reported higher levels of neopterin in many diseases including tuberculosis and cancer (6, 7). But these studies were carried out outside Nigeria. The present study was designed to bridge this gap in knowledge by evaluating the changes in the levels of albumin (ALB), total antioxidant potential (TAP), catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), total plasma peroxide (TPP), oxidative stress index (OSI), malondialdehyde (MDA), neopterin and nitric oxide (NO) in Nigerian PTB patients.

MATERIALS AND METHODS

Materials

Thirty-eight (17 males and 21 females) newly diagnosed Nigerian PTB-patients participated in this study. Diagnostic criteria included sputum acid fast bacilli (AFB) test, Mantoux test and radiological examination. The PTB patients presented with weight loss, chest pain, prolonged tiredness, loss of appetite and persistent cough for over three weeks. The radiological examination (chest X-ray) showed pulmonary inflammation. Many of the PTB patients already had blood stained sputum and shortness of breath by the time they were recruited for this study. Forty (22 males and 18 females) apparently healthy non-PTB individuals who were negative to Mantoux test served as controls. All participants were negative to HIV-antibodies that could interfere with the results of this study. Ethical approval was obtained from Damien Foundation of Belgium, Nigeria Chapter

Table 1*. Physical parameters in PTB-patients and non-PTB controls.*

	n	*Age (years)*	*Height (m)*	*Weight (Kg)*	*BMI Kg/m²*
PTB patients	*38*	*33.5+12.0*	*1.60+0.06*	*48.0+10.0*	*18.6+3.4*
Controls	*40*	*35.3+12.5*	*1.64+0.06*	*63.9+11.2*	*23.7+2.6*
t value,		*1.3,*	*1.7,*	*4.5,*	*4.7,*
p-values		*0.2*	*0.1*	*0.00**	*0.00**

*n: number of subjects, *: significantly different from the controls.*

(a non-governmental organization funding the eradication of tuberculosis and leprosy in Oyo and Osun states of Nigeria), through the Oyo State Ministry of Health, Nigeria. Ten milliliters (10ml) of blood was withdrawn from each participant. 2ml of the blood was put in EDTA bottle, out of which 0.5 ml was washed in physiological saline for the SOD assay. The remaining 8 ml of the blood for ALB, TAP, CAT, GSH, TPP, OSI, MDA, neopterin and NO was put into a lithium heparin container, and the plasma separated immediately. The plasma sample was stored at -200C until ready for analysis.

Estimation of TPP, OSI and MDA, NO and neopterin

TPP was determined using FOX-2 method (8), while OSI was estimated by calculating the percent ratio of the total plasma peroxide to the total antioxidant potential (8). MDA was determined using the method described by Vashney and Kale (9). NO was estimated using the method described by Wanchu et al (10), neopterin was determined in the plasma using a commercially prepared reagent, Neopterin ELISA kit (RE59321) by IBL Hambunrg, as described by Smith et al (11). The chemicals and standards for the preparation of reagents for the assay of TPP, OSI, MDA and NO were purchased from Aldrich Chemical Company, Inc., USA.

Estimation of TAP, ALB, SOD, GSH and CAT

TAP was estimated using the ferric reducing / antioxidant power (FRAP) assay (12, 13). The ALB concentration was determined by using a commercially prepared reagent (bromo cresol green solution) purchased from Dialab Production and Vertrieb vonchemich-technischen, Wien- Panikengasse. SOD was estimated with a colorimetric method described by Suttle (14), using a commercially prepared reagent manufacturer by Randox Laboratories Ltd, Antrim, UK. The GSH was determined by the method described by Beutler, et al (15), while CAT activity was determined by the method of Sinha (16), modified by Nwanjo (17). The chemicals and standards for the preparation of reagents for the assay of TAP, GSH and CAT were purchased from Aldrich Chemical Company, Inc., USA.

Statistical analysis

Data processing and statistics were done using SPSS version 10. The data were normally distributed and were expressed as means ± SD. Student (t) test was used for comparison of schizophrenics and controls. The changes were considered significant, when p-values were less than 0.05. Correlation of data was not done in this study.

RESULTS

Table 1 shows the physical characteristics of PTB patients and non-PTB controls during the study. There were no significant differences in the age and height at any

Table 2*. Levels of TPP, OSI, MDA, neopterin and NO in PTB patients and controls.*

	n	*TPP (µmol H_2O_2/L)*	*OSI (%)*	*MDA (nMol/ml)*	*Neopterin (nMol/ml)*	*NO (µmol/L)*
PTB-patients	*38*	*41.3+11.0*	*4.2+1.6*	*8.4+1.7*	*26.5+8.0*	*8.4+7.0*
Controls	*40*	*14.3+6.4*	*0.74+0.4*	*6.15+1.9*	*11.4+5.0*	*14.9+9.6*
t value		*3.3*	*6.9*	*3.3*	*2.3*	*2.2*
p-values		*0.00**	*0.00**	*0.00**	*0.02**	*0.04**

**: significantly different from the control, n: number of subjects*

***Table 3**. Levels of antioxidant indices in PTB patients and controls.*

	n	TAP (µmol Trolox equiv./L)	ALB (g/dL)	SOD (u/ml)	GSH (ng/ml)	CAT (µMol/L H_2O_2)
PTB-patients	38	1000+410	3.6+0.5	127+30	18+8.0	56+16
Controls	40	1540+430	4.8+0.4	201+26	26.3+10	72+16
t		2.8	7.5	7.4	2.7	2.6
p-values		0.01*	0.00*	0.00*	0.04*	0.04*

**: significantly different from the control, n: number of subjects*

point during the study. When compared with the control group, the weight and body mass index (BMI) decreased significantly in the PTB patients (p< 0.00). Table 2 shows the mean values of TPP, OSI, MDA, NO and neopterin in PTB-patients and controls. All PTB-patients showed significantly higher levels of TPP, OSI, MDA and neopterin when compared with the controls. But the mean level of NO decreased significantly in PTB-patients when compared with the controls. As shown in Table 3, the mean levels of TAP, ALB, SOD, GSH and CAT decreased significantly when compared with the controls.

DISCUSSION

In health, the free radicals generated from normal metabolic activities are controlled at physiological levels by the antioxidant system. Several reports show that excessive generation of reactive oxygen species (ROS) and RNI in pulmonary tuberculosis is the consequence of hyper-activated macrophages (20, 21). In the present study, the mean values of markers of oxidative stress (TPP, MDA and OSI) were significantly higher when compared with the controls. This finding confirmed the earlier studies by Wiid et al (18) and Akiibinu et al (19) that levels of free radicals are higher in PTB patients. The report of Kwiatkowska et al (20) showed that the mean levels of MDA and other products of lipid peroxidation in PTB patients were significantly higher than those of a healthy control group. The significantly higher level of TPP in this study could be associated with inadequate intake of antioxidant micronutrients and excessive production of free radicals by the tubercle bacilli invaded macrophages. Since oxidation of polyunsaturated fatty acids (lipid peroxidation) is the consequence of high free radical load and oxidative stress, the significantly higher value of MDA (a marker of lipid peroxidation) in this study may be due to higher levels of TPP and OSI in the PTB-patients.

The PTB patients recruited for this study showed significantly lower levels of antioxidant indices (TAP, ALB, SOD, GSH and CAT). The TAP is an index of all classes of antioxidants while the antioxidant enzymes (SOD, GSH and CAT) are trace-metal dependent. The lower levels of TAP, SOD, GSH, and CAT in our PTB patients could therefore be due to either the exhaustion of the antioxidant enzymes in the process of neutralization of the high free radical load or micronutrients deficiency (23). The significantly lower activity of markers of antioxidant system in our PTB patients is consistent with the report of previous workers (5, 18, 24 and 25) who reported significantly lower levels of classes of antioxidants in tuberculosis. Sasaki et al (26) stated that albumin and total protein were significantly lower in pulmonary tuberculosis. Aily et al (27) also observed lower levels of albumin and heamatocrite in tuberculosis. Since albumin is an antioxidant, the significantly lower level in our PTB patients could be due to its exhaustion during anti-oxidative activities.

Whereas our study demonstrated significantly lower level of NO in PTB patients, previous researchers have reported contradictory reports. Wanchu et al (10) reported significantly higher level of NO in tuberculosis and HIV-infection. Meanwhile, Schon et al (28) claimed that there was no significant difference in the level of NO observed in tuberculosis patients when compared with the controls. NO is a molecule formed in the cells through the conversion of the amino acid, L-arginine to NO by the action of nitric oxide synthase (NOs). Since L-guanine is the precursor of NO, malnutrition (commonly encountered in PTB) could contribute to the lower rate of synthesis of NO in PTB patients. The synergic effects of impaired synthesis and exhaustion of NO during anti-oxidative activities (29, 30 and 31) could therefore contribute to its significantly lower level in our PTB patients. Diversion of the NO to the peroxynitrite (ONOO) pathway could also contribute to the significantly lower level of NO in these PTB patients (32, 33). Since vitamin C regulates the local antioxidant capacity and maintains the physiological level of NO by enhancing its stability (34), significantly lower level of vitamin C already reported in the early part of this study could as well contribute to the lower level of NO in the PTB patients.

Neopterin is a marker of macrophage activation during infection. It is also a marker of the extent of oxidative stress elicited by the immune system (22). In the present study, the mean level of neopterin was significantly higher in PTB patients when compared with the controls. This result agrees with several other reports which demonstrated higher levels of neopterin in PTB patients. Chandara et al (6) reported higher neopterin concentration in TB co-infected HIV patients and in healthy health care workers, who had latent M. tuberculosis infections. Since high level of neopterin production has been associated with increased production of reactive oxygen species, the significantly higher level of neopterin in this study may be a consequence of excessive macrophage activation and excessive free radical generation in the PTB patients.

In conclusion, PTB patients exhibit oxidative stress and higher plasma level of neopterin. Since most of the antioxidants regulating the free radical load and oxidative stress are micronutrient dependent, micronutrient supplementation may be required as adjuvant therapy in the management of PTB patients.

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Authors' contributions: This study is a part of the Ph.D work by MOA. MOA took part in the designing of the study, did the sample analysis and the writing of the manuscript, EOO and EOS took part in the designing of the study and read through the manuscript.

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